FULL PAPER *Avian Pathology*

The Efficacy of Artesunate, Chloroquine, Doxycycline, Primaquine and a Combination of Artesunate and Primaquine against Avian Malaria in Broilers

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ABSTRACT. The efficacy of 5 antimalarial drugs was evaluated on *P. gallinaceum* infected broilers. One hundred and forty-seven 19-day-old broilers were divided into 7 groups of 21 chicks each. Group 1 was the unmedicated, uninfected control. Groups 2–6 were infected and medicated with artesunate, chloroquine, doxycycline, primaquine and an artesunate-primaquine combination, respectively. Group 7 was the unmedicated, infected control. Infectivity, mortality, parasitemia, schizonts in tissues and body weight gain were monitored. The results revealed that the two most effective drugs for treating *P. gallinaceum* at the asexual erythrocyte stage were chloroquine and doxycycline. Tissue schizonts of *P. gallinaceum* in all the medicated groups were significantly fewer than the unmedicated, infected control ($P<0.05$). The mortality rate of all the medicated groups was significantly lower than the unmedicated, infected control ($P<0.05$).
KEY WORDS: antimalarial drug, avian malaria, broilers, *Plasmodium gallinaceum*.

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Avian malaria is an avian disease caused by the *Plasmodium* protozoan. The disease agent has been classified as *Plasmodium gallinaceum* [12]. The parasite needs 2 hosts to complete its life cycle; chickens and mosquitoes. The sexual development of the parasite, called fertilization and sporogony, takes place in a female mosquito host, resulting in the production, at its infective stage, of sporozoites which reside in the salivary glands. Once an infected female mosquito bites a chicken, the malaria sporozoites are released and invade the reticuloendothelial cells as well as the erythrocytes and undergo asexual development known as schizogony. This stage takes place repeatedly, resulting in the host cell rupturing in many organs as well as in the blood cells. Parasite multiplication at this stage is responsible for the clinical signs of multiple organ failure, anemia or eventually, death. The parasites later develop into the next stage called gametogenesis to produce male and female gametocytes and are ready for the mosquito vectors to transmit the parasites to the next host. The competent mosquitoes for avian malaria are in the subfamily Culicinae, for instance, *Culex* spp., *Aedes* spp., *Mansonia* spp. and, rarely, *Anopheles* spp. [1].

Avian malaria has been reported in many countries in South east and East Asia including the Philippines, India, Indonesia, Sri Lanka, Malaysia and Vietnam. In Thailand, Mahantachaisakul *et al*. [7] reported the first cases of *P. gallinaceum* infection in broilers between August and October, 1995. The morbidity and mortality rates were 50–55% and 11–20%, respectively. In 1999, avian malaria was found in Siamese-Japanese mixed breed chickens in Khonkaen province between November 1998 and February 1999. The morbidity and mortality rates were 65% and 18%, respectively [5]. The clinical signs of infected chickens vary from no clinical signs to severe clinical signs. The mortality rate also varies depending on the species, age of the host and strain of *Plasmodium* [3]. Native chickens are more resistant to infection than commercial chickens. Infected chickens show variations of body temperature and anemia, and the mortality rate can be higher than 80% [15].

Avian malaria outbreaks do not frequently occur in Thailand. A few cases have been discovered during the rainy season, even though mosquito control strategies have been implemented. Therefore, an efficacy study of antimalarial drugs is still necessary to control and prevent the transmission of the disease. Antimalarial drugs are classified, based on the mechanism of action that is specifically active at certain developmental stages of *Plasmodium*. Classification of the mechanism of action can be divided into 3 groups; 1) tissue schizonticides which are active at the exoerythrocytic stage, such as proguanil, tetracycline and primaquine, 2) blood schizonticides which are active on the schizont located inside of erythrocytes, such as quinine, chloroquine and artemisinin and 3) gametocides which are active on the gametocytes in erythrocytes, such as primaquine [13]. The aim of this study is to assess the efficacy of the antimalarial drugs, artesunate, chloroquine, doxycycline and a combination of artesunate and primaquine to treat malarial infection in poultry. Artesunate and a combination of artesunate and primaquine are the first to be assessed in avian malaria treatment.

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MATERIAL AND METHODS

Broilers: Five hundred and ten, one day old, mixed sex, broiler chicks (Cobb Vantress 500) were obtained from a commercial hatchery (Sahafarm). The chicks were raised in isolated rooms at the Avian Health Research Unit, Chulalongkorn University. Feed and water were provided *ad libitum*. The guidelines and legislative regulations of Chulalongkorn University, Bangkok, Thailand on the use of animals for scientific purposes were followed.

P. gallinaceum isolate: *P. gallinaceum* isolate, namely MNTH 2543 from the Veterinary Parasitology Unit, was infected and maintained in layers. Blood with a higher parasitemia than 50% was used to inoculate, intravenously, 510 broilers at 14 days of age with an inoculum containing 1.22×10^8 infected erythrocytes per bird. Five days after infection, blood was collected, and a 10% Giemsa stained blood smear was performed and observed under a light microscope to evaluate parasitemia. The schizonts and gametocytes were monitored based on their distinct morphology. Infected broilers were selected and used for the antimalarial drug treatment.

Experimental designs and antimalarial drugs: One hundred and twenty six, infected broilers which had schizonts and gametocytes in their erythrocytes were randomly selected and divided into 6 groups (groups 2–7) of 21 chickens each. Extra 6 birds were raised in each group for the study of average schizonts in the endothelial cells of the liver, spleen, kidneys and brain at 4 days post treatment (DPT). At 19 days old, the chickens in groups 2–6 were given an oral application, once a day, for 5 days, of artesunate (Government Pharmaceutical Organization, Bangkok, Thailand), chloroquine (Shanghai International Pharmacy, Shanghai, China), doxycycline (Shanghai International Pharmacy), primaquine (Government Pharmaceutical Organization) and artesunate+primaquine (Government Pharmaceutical Organization), respectively, in doses of 10, 10, 50, 0.50 and 10+0.5 mg/kg body weight/day, respectively. The chickens in group 7 served as an infected, unmedicated control (IUC). Twenty one, healthy uninfected chickens serving as an uninfected, unmedicated control (UUC) (group 1) were given an oral application of phosphate buffered saline. Extra 6 birds were raised for the study of average schizonts in the endothelial cells of the liver, spleen or brain at 4 DPT.

Parameters monitored and data analyses: The efficacy of the antimalarial drugs was evaluated before and after treatment. After starting medication, all the birds were monitored for 10 days; 0–9 DPT. The observed parameters were infectivity, mortality, parasitemias, development of schizonts in the endothelial cells of the liver, spleen, kidneys and brain of 6 euthanized chickens at 0, 4 and 9 DPT and body weight gain during 0–9 DPT. Parasitemias were assessed by the examination of Giemsa-stained blood smears, and the results were shown as percentages of the erythrocytes infected [15]. One thousand erythrocytes were observed under a light microscope, and the percentage of parasitemia was calculated with the formula; number of infected erythrocytes/1,000 × 100. Histology was performed to investigate the schizonts of the endothelial cells in the liver, spleen, kidneys and brain by light microscopy. The schizonts were measured in 10 mm^2 of infected tissues by grid ocular micrometer, and the average schizont/mm^2 was calculated [4]. Infectivity and mortality were analyzed by χ^2. Schizonts and body weight gain were analyzed by ANOVA and the Duncan multiple range test by SPSS for Windows.

RESULTS

Mortality and infectivity: No morbidity or mortality was found in the UUC group 1. The mortality rate of chickens in the IUC group was 85.71% (Table 1). The mortality rate of chickens medicated with artesunate, chloroquine, doxycycline and artesunate+primaquine ranged from 23.81 to 28.57% which was significantly lower than the IUC group ($P<0.05$) (Table 1). The lowest mortality rate was 4.76% for chickens medicated with primaquine. The lowest infectivity was 66.67% for chickens medicated with chloroquine. Chickens medicated with doxycycline and artesunate+primaquine revealed 90.48% infectivity, while chickens medicated with artesunate and primaquine had 100% infectivity. The chickens medicated with chloroquine displayed the significantly lowest infectivity ($P<0.05$).

Average percentage parasitemias: No parasitemia was found in the UUC group. Chickens in the IUC group revealed an average parasitemia from 13.2 ± 11.4 to 63.7 ± 39.6% (Table 2). At 1 and 2 DPT, the average parasitemia of chickens medicated with artesunate was reduced from 3.81 ± 13% to 0.1 ± 0.4%, respectively compared to 19.7 ± 18.4% at 0 DPT followed by negative parasitemia at 3 DPT. At 4, 5 and 6 DPT, the average parasitemia was lower than that with average parasitemia before treatment. At 7–9 DPT, the average parasitemia was higher than that with prior treatment. The average parasitemia of chickens medicated with chloroquine was lower than 1.0% at 1 and 2 DPT followed by negative parasitemia at 3 and 4 DPT. The average parasitemia of chickens medicated with doxycycline increased during 0–3 DPT. At 5 DPT, the average parasitemia had been significantly reduced ($P<0.05$) compared to 0 DPT until the end of observation. The average parasitemia of chickens medicated with primaquine at 0–2 DPT was not different, but during 3–8 DPT, it was significantly reduced ($P<0.05$). At 9 DPT, parasitemia rebounded to a level similar to 0 DPT. The average parasitemia of chickens medicated with artesunate+primaquine was significantly reduced at 1 DPT ($P<0.05$) and was not found at 2 DPT. At 3–6 DPT, parasitemia was lower than 1.0%. At 7 DPT, parasitemia was increased to 2.3 ± 3.4%. At 8–9 DPT, the average parasitemia had rebounded to a level similar to 0 DPT.

Schizonts in the endothelial cells: The efficacy of antimalarial drugs was evaluated by the average of schizonts of *P. gallinaceum* in the endothelial cells of the liver, spleen, kidneys and brain comparing before and after treatment. No schizont was found in the endothelial cells of any observed organs of chickens in the UUC group throughout the monitoring period. This was contrary to the chickens in the IUC group 7, where schizonts were found in endothelial cells of

Table 1. Accumulated mortality rate and infectivity of broilers observed for 10 days after medication

Groups	Treatment	Accumulated mortality rate (birds)		Accumulated infectivity rate (birds)	
		Dead birds /total birds	%	Infected birds /total birds	%
1	none (UUC)	0/21	0.0^{a}	0/21	0.0^{a}
2	artesunate	5/21	23.81^{b}	21/21	100.0^{c}
3	chloroquine	6/21	28.57^{bc}	14/21	66.67^{b}
4	doxycycline	5/21	23.81^{b}	19/21	90.48^{c}
5	primaquine	1/21	4.76^{b}	21/21	100.0^{c}
6	artesunate+primaquine	5/21	23.81^{b}	19/21	90.48^{c}
7	none (IUC)	18/21	85.71^{d}	21/21	100.0^{c}

UUC=uninfected, unmedicated control; IUC=infected, unmedicated control. Different superscript in each column indicates statistical significance (P<0.05).

Table 2. Average% parasitemia of broilers (mean ± SD) after infection with *P. gallinaceum* before and after medication

Treatment	% parasitemia (DPT)									
	0 DPT	1 DPT	2 DPT	3 DPT	4 DPT	5 DPT	6 DPT	7 DPT	8 DPT	9 DPT
none (UUC)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
artesunate	19.7±18.4^{b}	3.8±13.0^{a}	0.1±0.4^{a}	0.0±0.0^{a}	0.1±0.2^{a}	0.5±0.7^{a}	5.4±5.5^{a}	21.4±23.4^{b}	54.9±32.6^{b}	59.8±30.1^{d}
chloroquine	21.8±18.4^{b}	0.4±0.7^{a}	0.3±0.6^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.4±0.6^{a}	0.6±1.2^{a}	1.6±1.8^{a}	1.7±3.2^{a}	0.9±1.8^{a}
doxycycline	18.1±20.0^{b}	50.1±38.7^{c}	46.5±35.8^{c}	23.9±19.1^{b}	9.0±11.3^{b}	1.7±1.7^{a}	1.41±1.50^{a}	1.1±1.1^{a}	4.4±8.0^{a}	7.1±11.6^{ab}
primaquine	20.7±14.7^{b}	35.6±18.1^{b}	25.0±16.0^{b}	2.4±2.3^{a}	0.4±0.6^{a}	0.9±0.8^{a}	1.0±0.8^{a}	2.2±1.6^{a}	11.7±12.1^{a}	23.5±21.7^{bc}
arte.+prim.	15.5±20.6^{b}	0.2±0.4^{a}	0.0±0.0^{a}	0.1±0.2^{a}	0.1±0.3^{a}	0.1±0.3^{a}	0.65±0.79^{a}	2.3±3.4^{a}	17.4±28.2^{a}	26.0±31.8^{c}
none (IUC)	13.2±11.4^{b}	56.1±36.8^{c}	63.7±39.6^{d}	47.0±34.4^{c}	45.0±32.1^{c}	50.7±42.5^{b}	37.8±39.1^{b}	36.3±32.9^{c}	42.0±18.3^{b}	55.7±19.4^{d}

DPT=day post treatment; UUC=uninfected, unmedicated control; IUC=infected, unmedicated control. Different superscript in each row indicates statistical significance (P<0.05).

Table 3. Average schizonts of *P. gallinaceum* (mean ± SD) in the endothelial cells of liver, spleen, kidneys and brain/mm^2 at 0, 4 and 9 day post treatment (DPT) of antimalarial treated groups, an uninfected, unmedicated control (UUC) and an infected, unmedicated control (IUC) groups

Treatments	schizonts in livers/1 mm^2			schizonts in spleens/1 mm^2			schizonts in kidneys/1 mm^2			schizonts in brains/1 mm^2		
	0 DPT	4 DPT	9 DPT	0 DPT	4 DPT	9 DPT	0 DPT	4DPT	9DPT	0 DPT	4DPT	9DPT
none (UUC)	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}
artesunate		10.4±7.2^{ab}	47.9±30.8^{c}		31.9±17.9^{b}	40.7±25.0^{b}		4.2±6.5^{a}	16.0±18.9^{a}		0.7±1.7^{a}	2.1±3.5^{ab}
chloroquine		17.9±6.3^{bc}	7.9±6.0^{ab}		33.7±8.1^{b}	17.7±6.6^{ab}		0.89±1.7^{a}	7.6±7.6^{a}		0.7±1.7^{a}	2.1±2.3^{ab}
doxycycline	9.6±10.2^{A}	28.8±17.9^{c}	13.3±13.7^{ab}	5.9±5.9^{B}	33.7±17.4^{b}	25.1±15.7^{ab}	11.1±7.8^{C}	44.4±49.3^{a}	11.1±15.7^{a}	9.7±10.8^{D}	3.5±4.1^{a}	0.0±0.0^{a}
primaquine		15.3±11.7^{b}	23.3±7.5^{b}		38.2±18.1^{b}	32.6±3.8^{b}		2.1±3.5^{a}	11.8±7.1^{a}		0.0±0.0^{a}	2.2±3.4^{ab}
arte.+prim.		12.4±7.1^{b}	18.3±22.0^{ab}		25.4±9.3^{b}	27.4±14.9^{b}		4.9±5.5^{a}	25.0±37.7^{ab}		0.0±0.0^{a}	9.0±14.0^{bc}
none (IUC)		54.6±10.3^{d}	51.0±17.2^{c}		71.8±33.9^{c}	98.7±42.4^{c}		87.5±83.1^{b}	50.7±43.2^{b}		7.6±5.5^{b}	12.5±7.5^{c}

A, B, C, D=average schizonts of *P. gallinaceum* (mean ± SD) in the epithelial cells at 0 DPT of all treated and IUC groups of liver, spleen, kidneys and brain/*1* mm^2, respectively. Different superscript in each column indicates statistical significance (P<0.05).

all observed organs throughout the observation period. At 4 and 9 DPT, the schizonts in the liver, spleen and kidneys were significantly higher than those at 0 DPT (P<0.05) (Table 3). In the brain, the number of schizonts at 0, 4 and 9 DPT was at a similar level. Schizonts in the endothelial cells of the liver, spleen, kidneys and brain were found in chickens medicated with various antimalarial drugs (groups 2–6). However, chickens medicated with doxycycline at 9 DPT and chickens medicated with primaquine and artesunate+primaquine at 4 DPT were found to have schizonts in the endothelial cells of the brain. Chickens medicated with various antimalarial drugs were found to have lower number of schizonts in the endothelial cells of the liver, spleen, kidneys and brain than the chickens in the IUC group.

Body weight gain: The average body weight of chickens before infection ranged from 522.86 ± 48.60 to 574.29 ± 34.14 g (Table 4). At 28 days old (10 days of observation), chickens in the UUC group had the highest average body weight gain (672.38 ± 90.11 g). On the other hand, chickens in the IUC group had the lowest average body weight gain (213.33 ± 95.04 g). The average body weight gain of chickens medicated with chloroquine, doxycycline and primaquine was significantly higher than that of chickens medicated with the artesunate, artesunate + primaquine and IUC groups (P<0.05).

Table 4. Average body weight of 21 chickens in each group (mean ± SD) before and after medication (termination) with antimalarial drugs

Treatment	Body weight (grams)		
	Before infection (19 days old)	Termination (28 days old)	Body weight gain
none (UUC)	574.29 ± 34.14^{d}	1246.67 ± 88.62^{d}	672.38 ± 90.11^{e}
artesunate	543.33 ± 39.79^{abc}	802.67 ± 128.26^{ab}	263.33 ± 112.99^{ab}
chloroquine	568.57 ± 35.54^{cd}	962.67 ± 126.01^{c}	386.67 ± 131.24^{cd}
doxycycline	562.86 ± 57.64^{cd}	997.50 ± 163.61^{c}	441.25 ± 137.69^{d}
primaquine	522.86 ± 48.60^{a}	968.57 ± 132.30^{c}	445.71 ± 111.78^{d}
arte.+prim.	555.24 ± 36.69^{bcd}	884.71 ± 110.35^{bc}	329.41 ± 117.87^{bc}
none (IUC)	530.00 ± 45.50^{a}	753.33 ± 90.19^{a}	213.33 ± 95.04^{a}

UUC=uninfected, unmedicated control; IUC=infected, unmedicated control. Different superscript in each column indicates statistically significance (P<0.05).

DISCUSSION

The efficacy of antimalarial drugs generally depends on activity at each stage of the parasites and their pharmacokinetics. After treatment with chloroquine or artesunate, the parasitemias were significantly reduced (P<0.05) (Table 2). According to the previous reports, that chloroquine and artesunate are only active against the schizonts in erythrocytes, but do not destroy them at the exoerythrocytic stage [6, 11]. One day after treatment with chloroquine, the parasitemias were significantly reduced (P<0.05). Chloroquine is well absorbed in the gastrointestinal tract and has a maximum plasma concentration within 3 hr. This drug acts rapidly to reduce parasitemia within 48–72 hr of application [14]. Therefore, the average parasitemia of infected chickens medicated with chloroquine, artesunate and artesunate+primaquine is rapidly reduced compared to the average parasitemia of infected chickens medicated with doxycycline and primaquine (Table 2). Also, after the end of chloroquine, artesunate or artesunate+primaquine treatment, the average parasitemia of these medicated groups gradually increased. This was in contrast to chickens medicated with primaquine and doxycycline, because these 2 drugs are mainly active against the schizont stage in the tissues, so the average parasitemia was gradually reduced during the course of medication [11]. In Thailand, Prasittirat *et al*. [9, 10] reported that the chloroquine and doxycycline were used to treat a naturally infection of avian malaria in broilers and layers.

Classical clinical manifestations followed by a high mortality rate among chickens infected with *P. gallinaceum* were developed throughout the course of infection. This high mortality rate has been recorded in many previous reports [2, 15]. The IUC group revealed the highest number of schizonts in the liver, spleen, kidneys and brain that accorded with the higher mortality rate in this group. In the liver, the number of schizonts in the endothelial cells in the IUC group was significantly higher than that of infected chickens medicated with other antimalarial drugs (P<0.05), except for infected chickens medicated with artesunate at 9 DPT. This means that the antimalarial drugs were successful in treating the infected chickens, especially during the course of treatment (0–4 DPT). At termination, the chloroquine treated group revealed a significantly lower number of schizonts in the endothelial cells of livers, spleen and kidneys compared to the IUC group (P<0.05). At 4 DPT, no schizonts were found in the brains of infected chickens medicated with primaquine and artesunate+primaquine, and the schizonts in all treated groups were significantly lower than those of the IUC group (P<0.05) (Table 3). In chickens infected with *P. gallinaceum*, schizonts could be found in the endothelial cell in the brain's capillaries. The pathogenesis of brain infection depends on the percentage of parasitemia and schizont development in the brain's endothelial cells. Lesions in the brain, including hemorrhage, congestion, swelling and schizonts, can cause brain dysfunction and neurological signs [3, 8]. At the end of the observation (28 days old), the average body weight gain of chickens in the UUC group was significantly highest in all the chickens in the infected chicken groups (P<0.05) (Table 4).

In the present study, artesunate, chloroquine, doxycycline, primaquine and artesunate+primaquine have been shown to display therapeutic activity against *P. gallinaceum* compared to the IUC group. However, chloroquine and doxycycline revealed better efficacy in treating infected chickens at the gametocyte stage than artesunate and artesunate+primequine.

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